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(54) Title: METHODS TO OBTAIN RECOMBINANT PROTEINS WITH INCREASED SIALYLATION FROM CELLS THAT EXPRESS ADENOVIRUS E1A PROTEIN, AND PROTEINS OBTAINED THEREBY

(57) Abstract: The invention provides compositions comprising one or more isoforms of an erythropoietin (EPO) comprising glycans linked thereto, characterized in that said glycans comprise LewisX structures and on average at least 6 sialic acid moieties per EPO molecule. The invention further provides methods for obtaining a composition comprising one or more isoforms of an erythropoietin (EPO) comprising glycans linked thereto wherein said glycans comprise on average at least 6 sialic acids per EPO molecule and from 0 to 2 Lewis x structures, said method comprising: a) providing a eukaryotic cell containing a nucleic acid sequence encoding an adenoviral E1A protein in expressible format and further containing a nucleic acid encoding an EPO in expressible format, wherein said cell further contains a nucleic acid sequence encoding a sialyltransferase, preferably an alpha-2,6-sialyltransferase or an alpha-2,3-sialyltransferase, under control of a heterologous promoter; b) culturing said cell in a serum-free culture medium and allow expression of an EPO in said cell; c) harvesting the expressed EPO from said cell and/or from the culture medium; and d) purifying and fractionating the EPO to obtain fractions which have an increased average sialic acid content of the N-linked glycans per EPO molecule, to obtain a composition comprising one or more iso forms of an EPO comprising glycans linked thereto wherein said glycans co mprise on average at least 6 sialic acids per EPO molecule and from 0 to 2 Lewis x structures.

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